HBC 204: METABOLISM I

LECTURE 4: MITOCHONDRIA STRUCTURE, ELECTRON TRANSPORT CHAIN & OXIDATIVE PHOSPHORYLATION

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Course Outline

WEEK	DATE	TOPIC
1	22.02	BIOENERGETICS: standard free energy in chemical reactions, exergonic and endergonic reactions; Standard free energy of hydrolysis of ATP, Enzymatic transfer of phosphate groups to ATP; Properties of. ATP and high energy phosphate compounds
2	01.03	**CARBOHYDRATE METABOLISM:** Carbohydrate digestion & mobilization; Glycolysis and Its regulations, Substrate Level Phosphorylation; pyruvate oxidation.
3	08.03	**KREBS CYCLE:** Krebs cycle and regulation; Anaploretic reactions; ~~phosphogluconate pathway.~~
4	15.03	**MITOCHODRIAL STRUCTURE & FUNCTION:** Electron Transfer Chain; Oxidative Phosphorylation; Mechanisms of ATP generation; Uncouplers; inhibitors of ATP generation
5	22.03	**DISACCHARIDE METABOLISM:** Phosphogluconate pathway; Glycogen metabolism; Glycogenolysis and gluconeogenesis; Regulation of glycogen metabolism; Covalent modification; cAMP and hormonal regulation; Glycogen storage disease
	29.03	• CAT I

Introduction

ATP is generated as a result of the energy produced when electrons from NADH and $FADH_2$ are passed to molecular O_2 by a series of electron carriers, collectively known as the electron transport chain. The components of the chain include **FMN, Fe–S centers, coenzyme Q**, and a series of **cytochromes (b, c1, c, and aa3).**

- The energy derived from the transfer of electrons through the ETC is used to **pump protons across the inner mitochondrial membrane from the matrix to the cytosolic side**. An electrochemical gradient is generated, consisting of a proton gradient and a membrane potential.

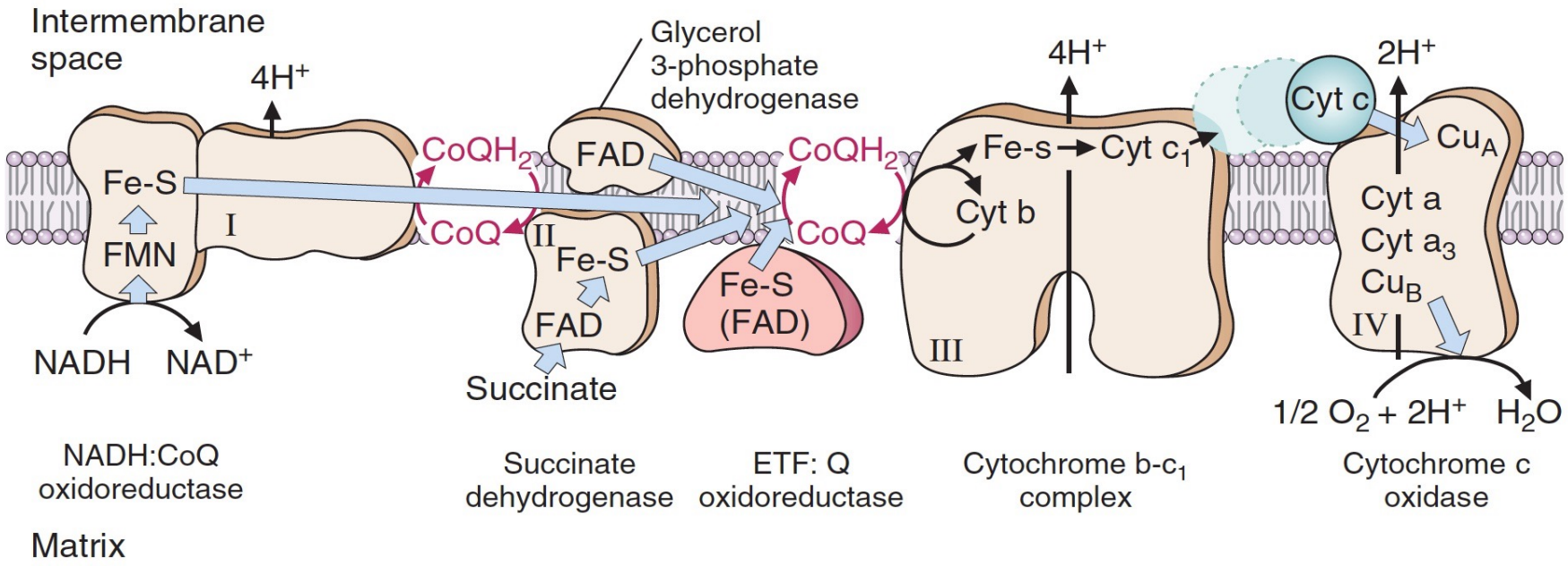
- **Protons move back into the matrix through the ATP synthase complex**, causing ATP to be produced from ADP and Pi.

- ATP is transported from the mitochondrial matrix to the cytosol in exchange for ADP (the ATP–ADP antiport system).

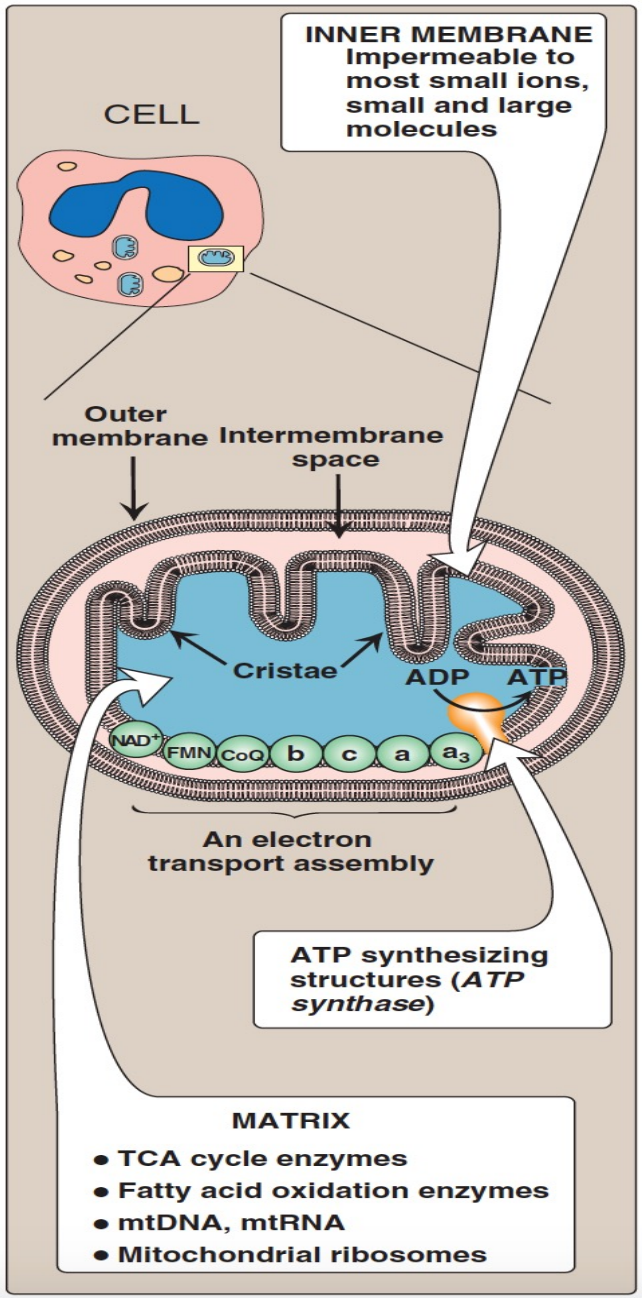
- The oxidation of **1 mole of NADH generates approximately 2.5 moles of ATP**, whereas the oxidation of **1 mole of FADH2 generates approximately 1.5 moles of ATP**.

- Because energy generated by the transfer of electrons through the ETC to O_2 is used in the production of ATP, the overall process is known as **oxidative phosphorylation**. ETC and ATP production occur simultaneously and are tightly coupled.

ELECTRON TRANSPORT CHAIN



Flow of electrons Components of the electron transport chain. Heavy arrows indicate the flow of electrons. CoQ, coenzyme Q (ubiquinone); Cyt, cytochrome; Fe–S, iron–sulfur centers; FMN, flavin mononucleotide.



Components of the electron transport chain

The reduced cofactors, NADH and FADH2, transfer electrons to the electron transport chain, which is located in the inner mitochondrial membrane. The chain consists of a number of protein complexes, designated as I through IV.

1. **FMN** receives electrons from NADH in complex I and transfers them through Fe–S centers to coenzyme Q. *** FMN is derived from **riboflavin (vitamin B2)**.

2. Coenzyme Q receives electrons from FMN and also through Fe–S centers from $FADH_2$ (such as complex II).

- $FADH_2$ is not free in solution like NAD^+ and NADH; it is tightly bound to enzymes.
- Coenzyme Q can be synthesized in the body. It is not derived from a vitamin.

3. Cytochromes in complex III receive electrons from the reduced form of coenzyme Q.

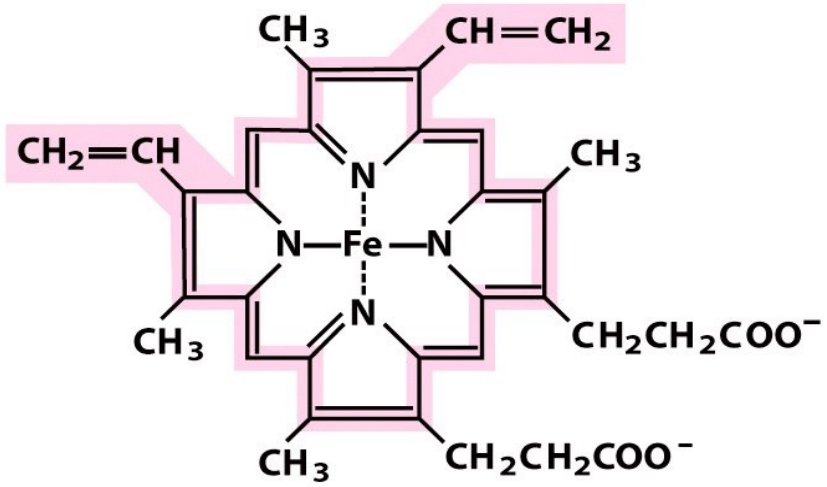
- Each cytochrome consists of a heme group associated with a protein.
- The iron of the heme group is reduced when the cytochrome accepts an electron.

$$Fe^{3+} \rightleftharpoons Fe^{2+}$$

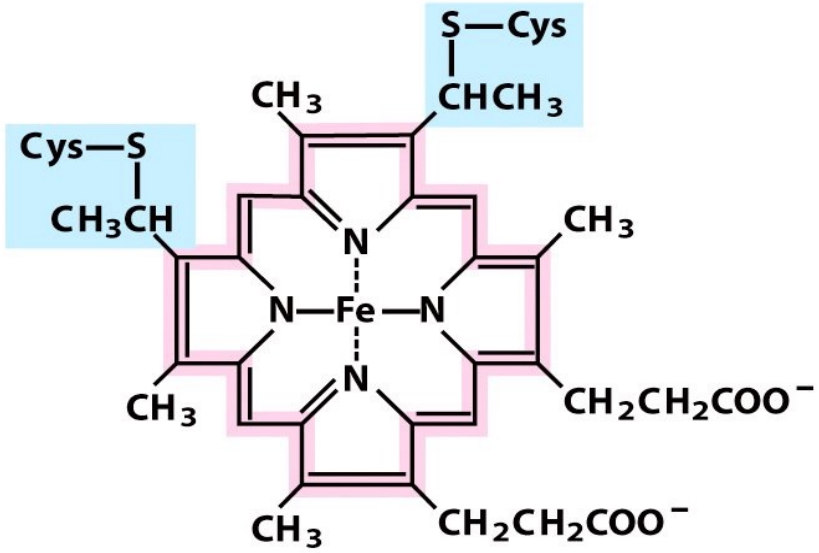
- Heme is synthesized from glycine and succinyl-CoA in humans. It is not derived from a vitamins.

4. Oxygen (O_2) ultimately receives the electrons at the end of the electron transport chain and is reduced to H_2O (a function of complex IV).

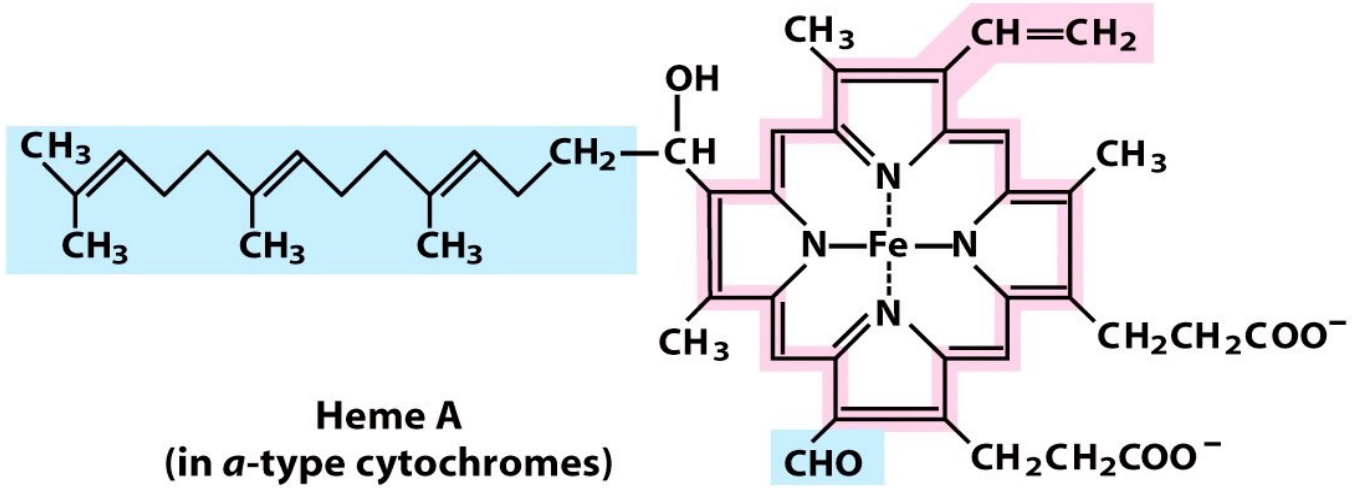
Heme groups



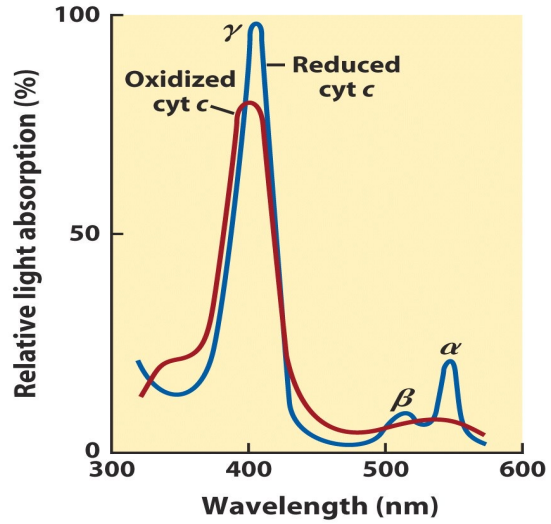
Iron protoporphyrin IX
(in *b*-type cytochromes)



Heme C
(in *c*-type cytochromes)



Heme A
(in *a*-type cytochromes)



The Mitochodria: Where ETC and OP occur

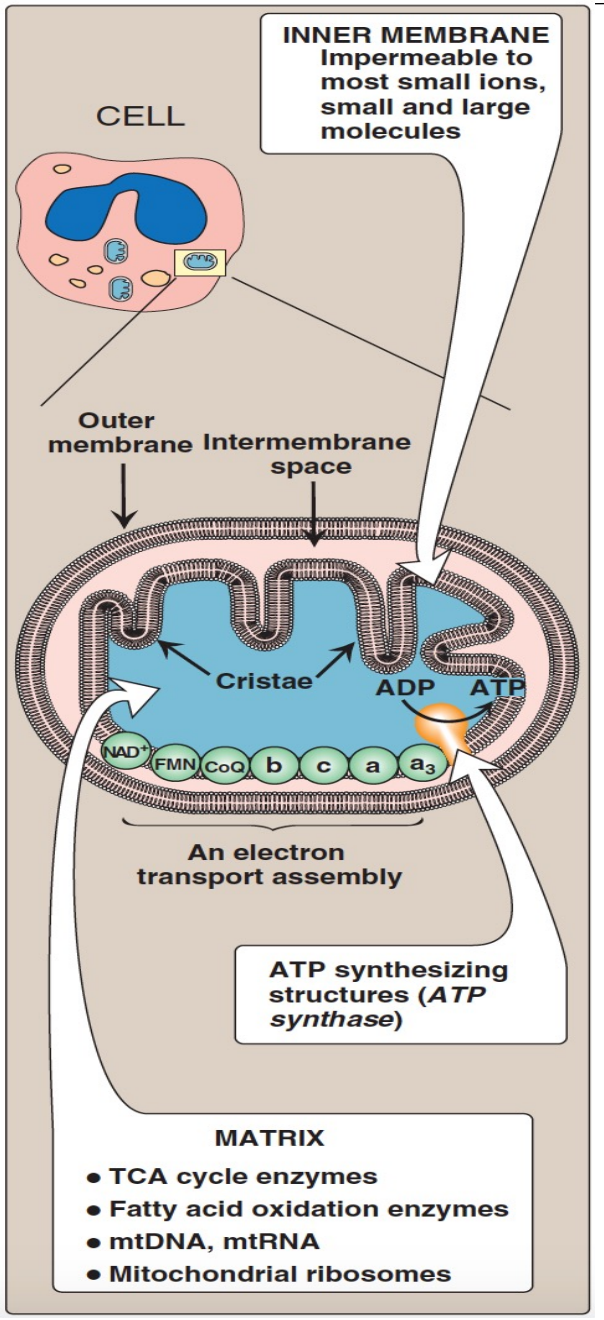
1. Mitochondria are separated from the cytoplasm by two membranes. The soluble interior of a mitochondrion is called the matrix. The matrix is surrounded by the inner membrane, which contains infoldings known as **cristae.**

- The **transfer of electrons from NADH to O_2** occurs in three stages, each of which involves a large protein complex in the inner mitochondrial membrane.
- Each complex uses the energy from electron transfer to **pump protons** to the cytosolic side of the membrane.
- An electrochemical potential or proton-motive force is generated.
  a. The electrochemical potential consists of both a membrane potential and a pH gradient.
  b. The cytosolic side of the membrane is more acidic **(i.e., has a higher [H^+])** than the matrix.

2. The inner mitochondrial membrane is impermeable to protons. **The protons can re-enter the matrix only through appropriate carriers. One of these is the ATP synthase complex** (the F_0-F_1/ATPase), **causing ATP to be generated.**

- The ATP synthase complex contains proteins (F_0) that form a channel in the inner mitochondrial membrane, through which the protons can flow, and a stalk that is attached to an ATP-synthesizing head (F_1) that projects into the matrix.

3. The electron transport chain has a large negative $\Delta G^{o'}$, and thus the electrons flow from NADH (or $FADH_2$) toward O_2.



3 major stages of electron transport

STAGE 1

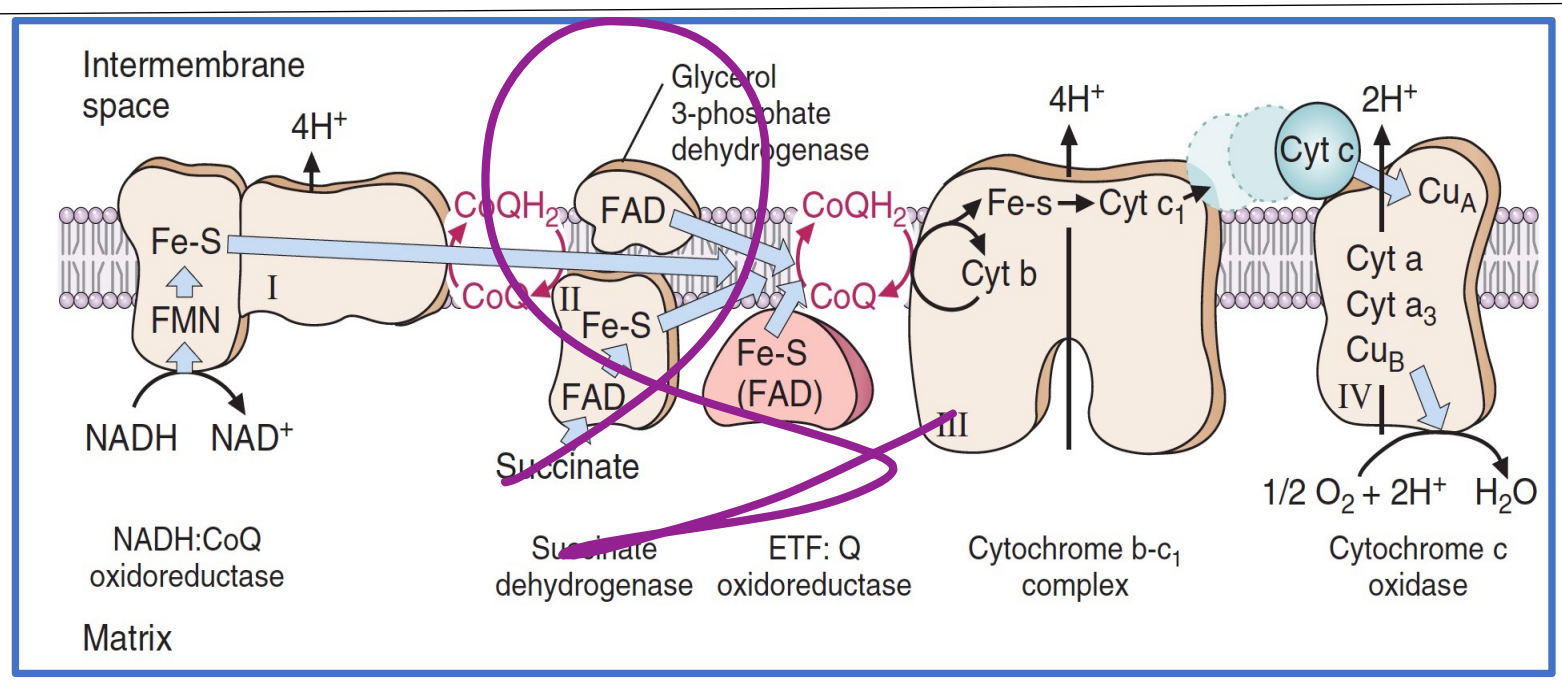
1. Transfer of electrons from NADH to coenzyme Q

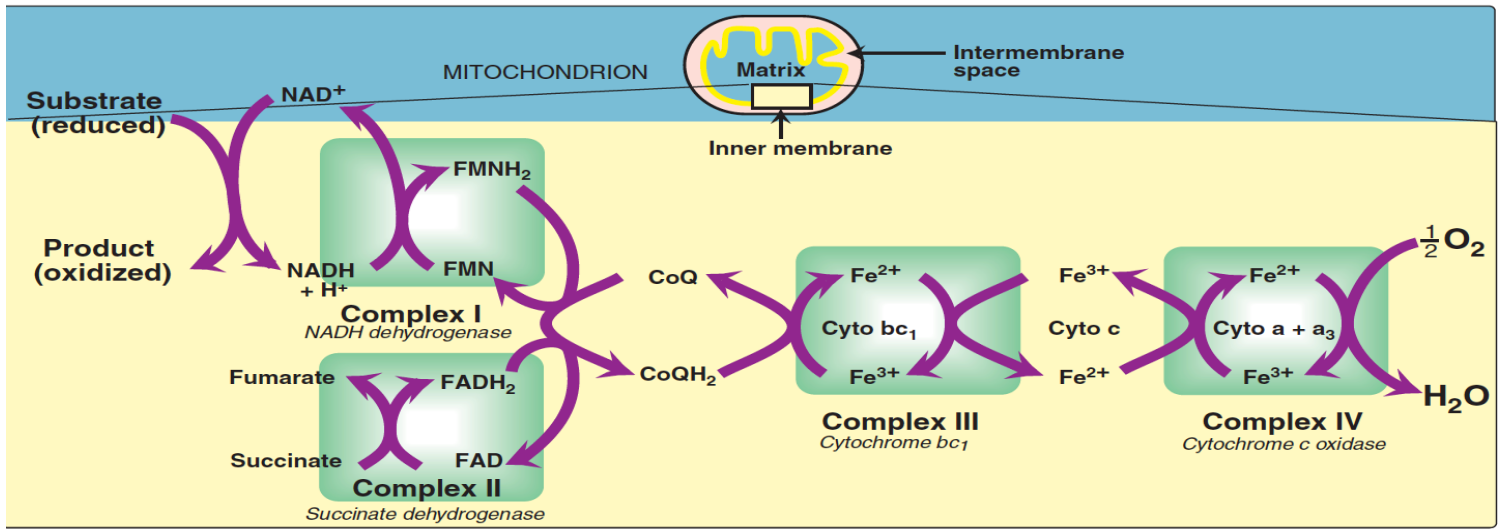
a. **NADH** passes electrons via the **NADH dehydrogenase complex (complex I)** to FMN. The complex is also known as the **NADH:CoQ oxidoreductase.**

b. **FMN** passes the electrons through a series of iron–sulfur (Fe–S) complexes to coenzyme Q, which accepts electrons one at a time, forming first the semiquinone and then ubiquinol.

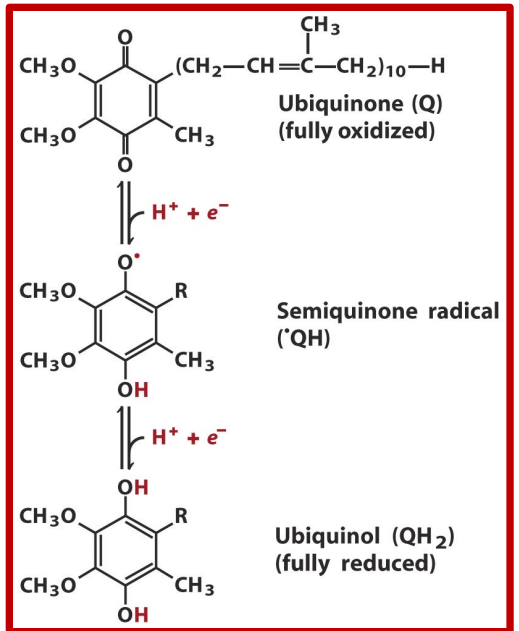
c. The energy produced by these electron transfers is used to pump protons to the cytosolic side of the inner mitochondrial membrane.

d. As the protons flow back into the matrix through the pores in the ATP synthase complex, ATP is generated.





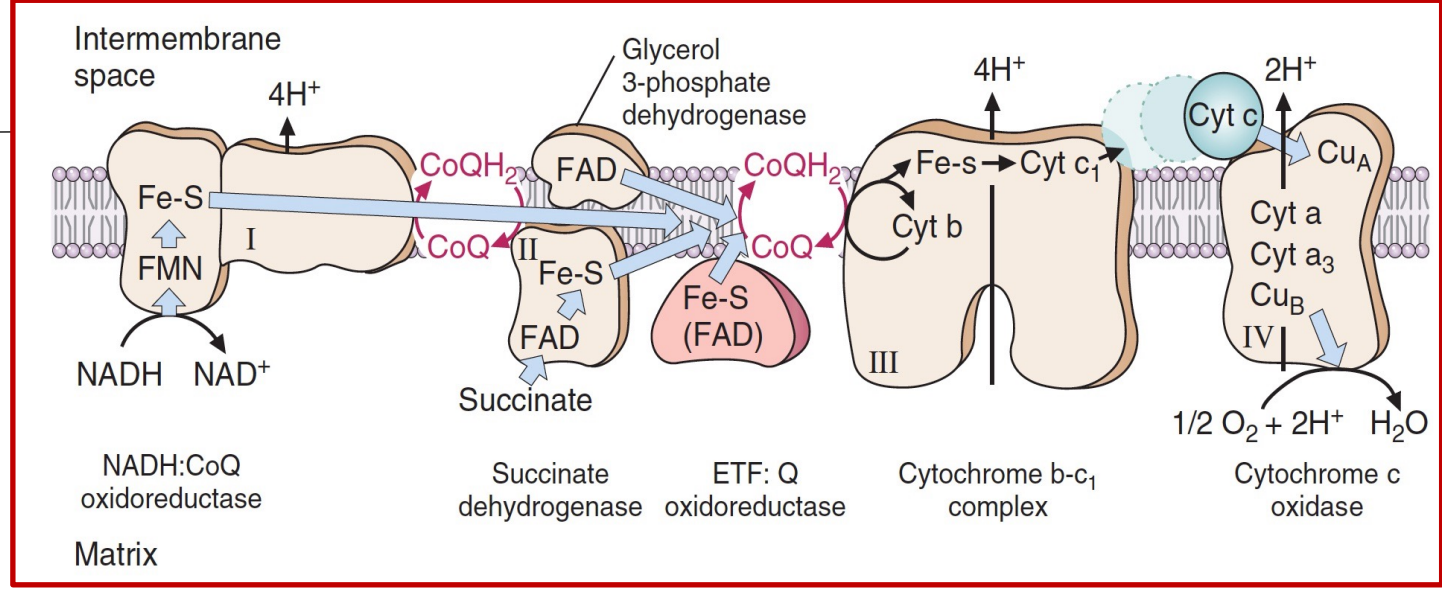
Coenzyme Q = ubiquinone



3 major stages of E.T.C

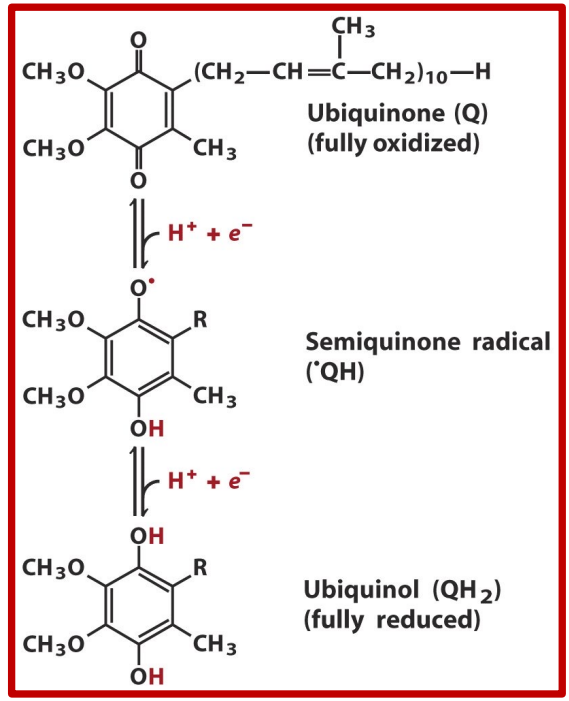
STAGE 2

2. Transfer of electrons from coenzyme Q to cytochrome c



a. **Coenzyme Q** passes electrons through Fe–S centers to **cytochromes b and c1**, which transfer the electrons to **cytochrome c**. The protein complex involved in these transfers is called **complex III**, or the cytochrome b-c1 complex. The complex is also known as **CoQ:C1 oxidoreductase**

- These cytochromes each contain heme as a prosthetic group but have different apoproteins.
- In the **ferric (Fe^{3+})** state, the heme iron can accept one electron and be reduced to the ferrous (**Fe^{2+}**) state.
- Because the cytochromes can only carry one electron at a time, two molecules in each cytochrome complex must be reduced for every molecule of NADH that is oxidized.

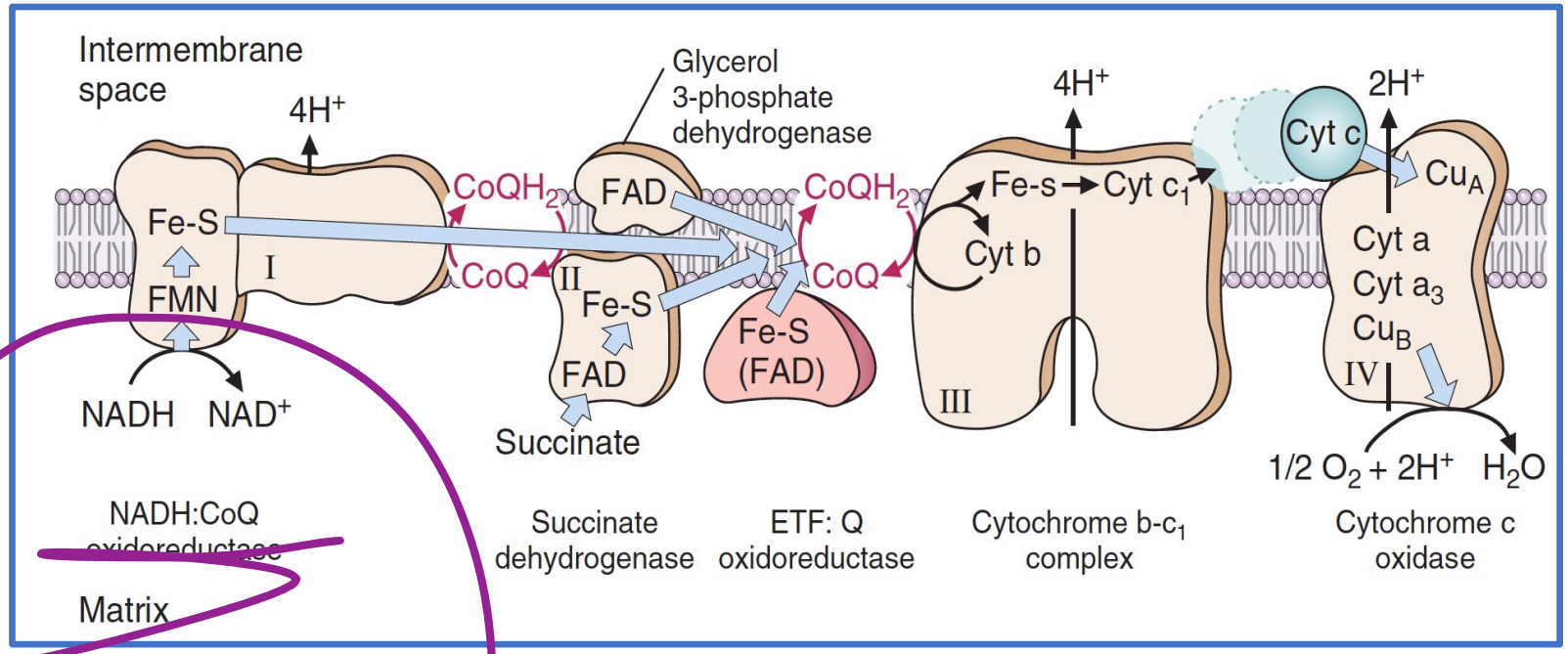


b. **Electrons from $FADH_2$**, produced by reactions such as the oxidation of succinate to fumarate, enter the electron transport chain at **complex II, which contains succinate dehydrogenase**. Complex II will transfer electrons to coenzyme Q, without the associated proton pumping across the inner mitochondrial membrane.

3 major stages of electron transport

STAGE 3

Transfer of electrons from cytochrome c to oxygen



a. **Cytochrome c** transfers electrons to the cytochrome aa_3 complex, which **transfers the electrons to molecular O_2, reducing it to H_2O. Cytochrome oxidase (complex IV)** catalyzes this transfer of electrons.
   - ➔ (1) Cytochromes a and a_3 each contain a heme and two different proteins that each contain copper.
   - ➔ (2) Two electrons are required to reduce one atom of oxygen; therefore, for each NADH that is oxidized, one-half of O_2 is converted to H_2O.

b. The energy produced by the transfer of electrons from cytochrome c to O_2 is used to pump protons across the inner mitochondrial membrane.

c. As the protons flow back into the matrix, ATP is generated.

ATP production

1. The production of ATP is **coupled** to the transfer of electrons through the electron transport chain to O_2. The overall process is known as **oxidative phosphorylation.**

2. Protons flow down their electrochemical gradient through the **membrane-bound ATP synthase**. The flow of protons through the ATPase allows the enzyme to synthesize ATP.

3. The exact amount of ATP that is generated by this process has not been unequivocally established, for each pair of electrons that enters the chain from NADH, 10 protons are pumped out of the mitochondria. As it takes four protons to flow through the ATPase to synthesize one ATP, 2.5 moles (10 divided by 4) of ATP can be generated from 1 mole of NADH.

    a. For every mole of **NADH** that is oxidized, 0.5 moles of O_2 is reduced to H_2O and approximately **2.5 moles of ATP** are produced.

    b. For every mole of **FADH2** that is oxidized, approximately **1.5 moles of ATP** are generated because the electrons from $FADH_2$ enter the chain via coenzyme Q, bypassing the NADH dehydrogenase step.

    *** Electrons entering via complex II lead to the extrusion of 6 protons per pair of electrons, instead of the 10 protons per pair of electrons starting at complex I. This is because complex II does not extrude protons as electrons flow through the complex.

The ATP–ADP antiport

- **ATP produced within mitochondria is transferred to the cytosol in exchange for ADP** by a transport protein in the inner mitochondrial membrane known as the adenine nucleotide translocase **(ANT).**

- The energy for their transport is the proton gradient, as ATP has four negative charges (leaving the matrix), and ADP contains three negative charges (entering the matrix).

Inhibitors of electron transport and oxidative phosphorylation

1. Agents that act on components of the electron transport chain

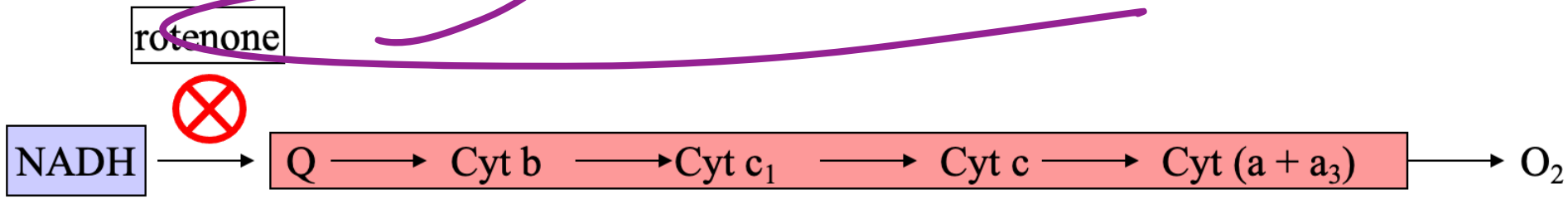
2. Inhibitors of ATP synthesis

3. Uncoupling agents

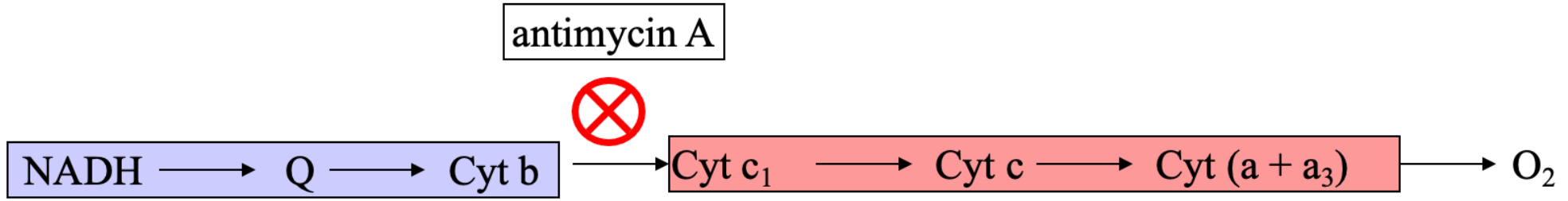
(i) Agents that act on components of the electron transport chain

❖ If there is a block at any point in the electron transport chain, all carriers before the block will accumulate in their reduced states, whereas those after the block will accumulate in their oxidized states. As a result, O_2 will not be consumed; ATP will not be generated; and the TCA cycle will slow down owing to the accumulation of NADH.

1. **Rotenone:** a fish poison, complexes with complex I, causing NADH to accumulate. It does not block the transfer of electrons to the chain from $FADH_2$.

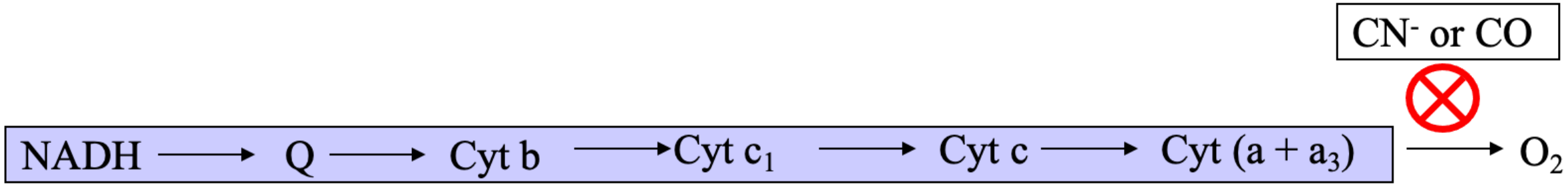


2. **Antimycins:** (antibiotic) block the passage of electrons through the cytochrome b-c_1 complex (complex III).



(i) Agents that act on components of the electron transport chain

3. Cyanide & Carbon monoxide: combine with cytochrome oxidase (complex IV) and block the transfer of electrons to O_2.



(2) Inhibitors of ATP synthesis

- Because the synthesis of ATP and electron transport are coupled, if the ATP synthase complex is inhibited or if an adequate supply of ADP is not available, ATP synthesis will be inhibited, O_2 will not be consumed, the components of the electron transport chain will accumulate in their reduced states, and the TCA cycle will slow down.

1. **Atractyloside:** will inhibit the adenine nucleotide translocase (ANT). Therefore, ATP synthesis will stop owing to a lack of ADP in the mitochondrial matrix.

2. **Dicyclohexylcarbodiimide(DCCD) & oligomycin** block the proton pore (F_0 component of the ATP synthase), preventing ATP synthesis and blocking oxidative phosphorylation.

(3) Uncoupling agents

❖ **Dinitrophenol:** These are ionophores that allow protons from the cytosol to re-enter the matrix without going through the pore in the ATP synthase complex. Thus, they uncouple electron transport and ATP production.

Uncouplers **increase the rate of O_2 consumption, electron transport, TCA cycle and CO_2 production**.

➔ The energy generated by the increased rate of respiration (electron transport and O_2 consumption) is lost as heat.

Clinical Correlates

CLINICAL CORRELATES

Iron-deficiency anemia is due to a lack of iron for heme synthesis, which will lead to reduced oxygen delivery to the tissues. This, in conjunction with reduced heme levels in the electron transfer chain due to the reduced iron levels, can lead to muscle weakness because of an inability to synthesize appropriate amounts of ATP.

CLINICAL CORRELATES

An acute myocardial infarction is due to a reduction in blood flow to a specific region of the heart. Coronary arteries frequently become narrow because of **atherosclerotic plaques**. If **coronary occlusions** occur, regions of heart muscle may be deprived of blood flow and, therefore, of oxygen for prolonged periods of time. **Lack of oxygen** causes inhibition of the processes of electron transport and oxidative phosphorylation, which results in a decreased production of ATP. **Heart muscle**, suffering from a lack of energy required for contraction and maintenance of membrane integrity, becomes **damaged**. **Enzymes** from the damaged cells (including the MB fraction of creatine kinase) **leak into the blood**. If the damage is relatively mild, the person may recover. If heart function is severely compromised, death may result.

Generation of ATP by glycolysis

Energy generated by conversion of glucose to CO_2 and H_2O

a. When glucose is oxidized completely to CO2 and H2O, approximately 30 or 32 moles of ATP are generated.

i. Two moles of ATP and 2 moles of NADH are generated from the conversion of 1 mole of glucose to 2 moles of pyruvate.

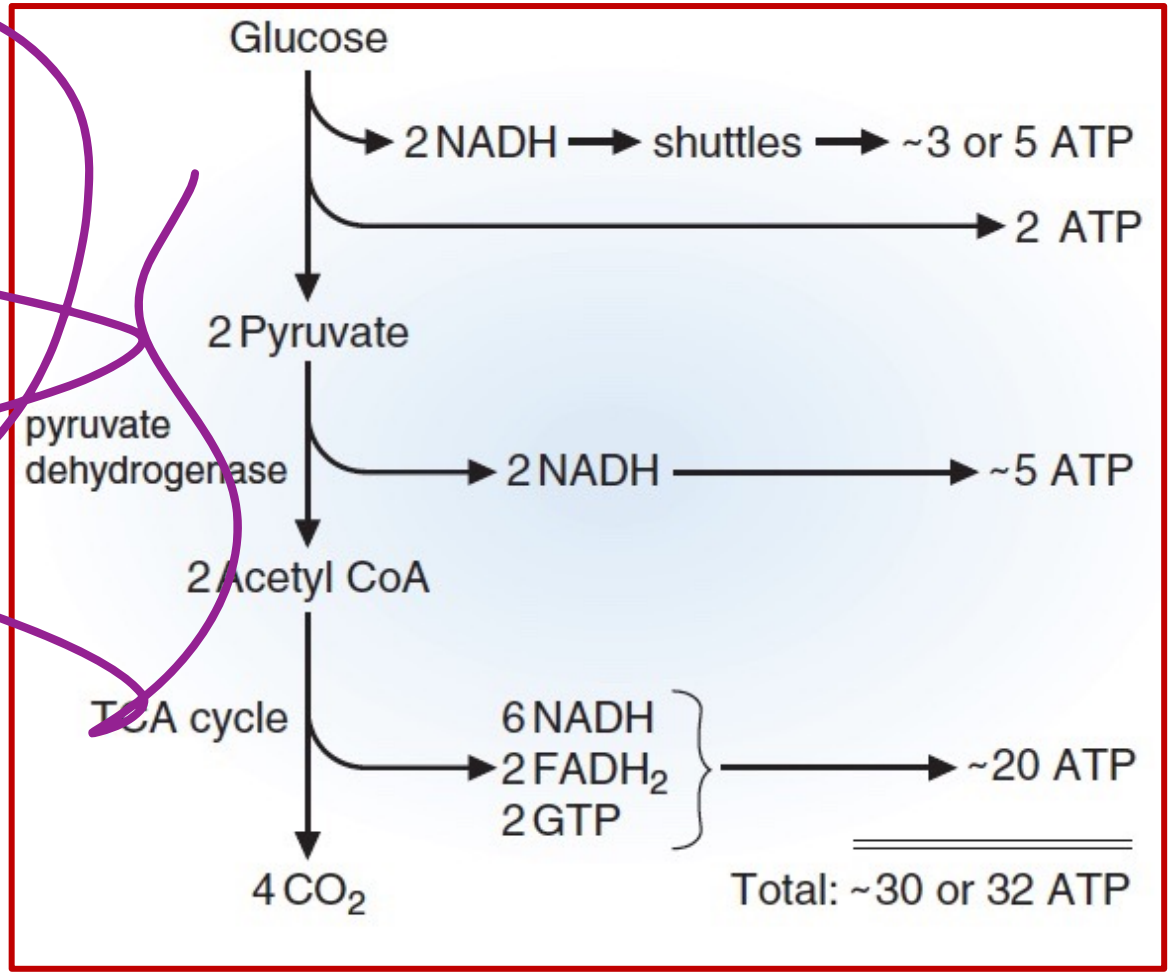
ii. The 2 moles of pyruvate enter the mitochondria and are converted to 2 moles of acetyl-CoA, producing 2 moles of NADH, which generate approximately 5 moles of ATP by oxidative phosphorylation.

iii. The 2 moles of acetyl-CoA are oxidized in the TCA cycle, generating approximately 20 moles of ATP.

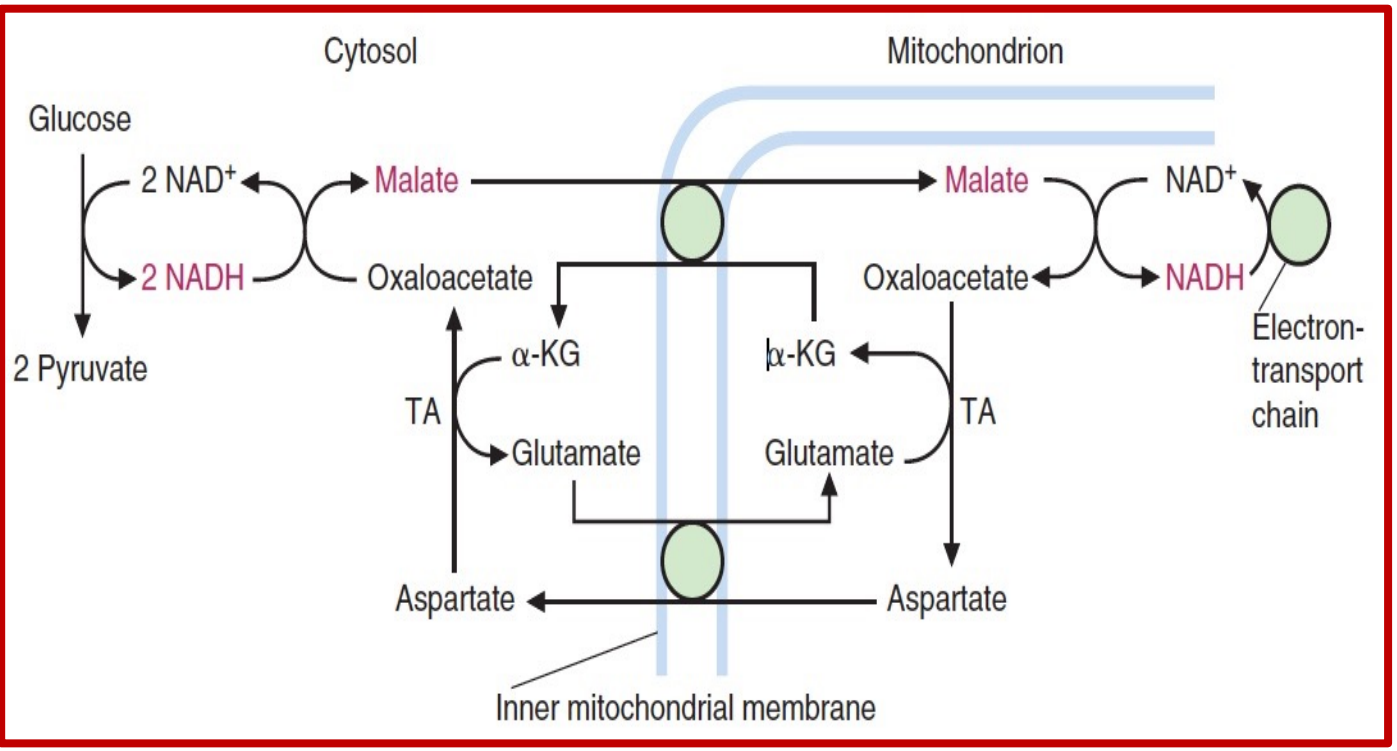
iv. NADH, produced in the cytosol by glycolysis, cannot directly cross the mitochondrial membrane. Therefore, the electrons are passed to the mitochondrial electron transport chain by two shuttle systems: Glycerol phosphate shuttle
Malate aspartate shuttle

Maximal ATP production

a. Overall, when 1 mole of glucose is oxidized to CO_2 and H_2O, approximately 30 moles of ATP are produced if the glycerol phosphate shuttle is used, or 32 moles if the malate aspartate shuttle is used.



NADH Shuttle system: Malate aspartate shuttle



a) Cytosolic oxaloacetate is reduced to malate by NADH. The reaction is catalysed by **cytosolic malate dehydrogenase.**

b) Malate enters the mitochondrion and is reoxidized to oxaloacetate by the **mitochondrial** malate dehydrogenase, generating NADH in the matrix.

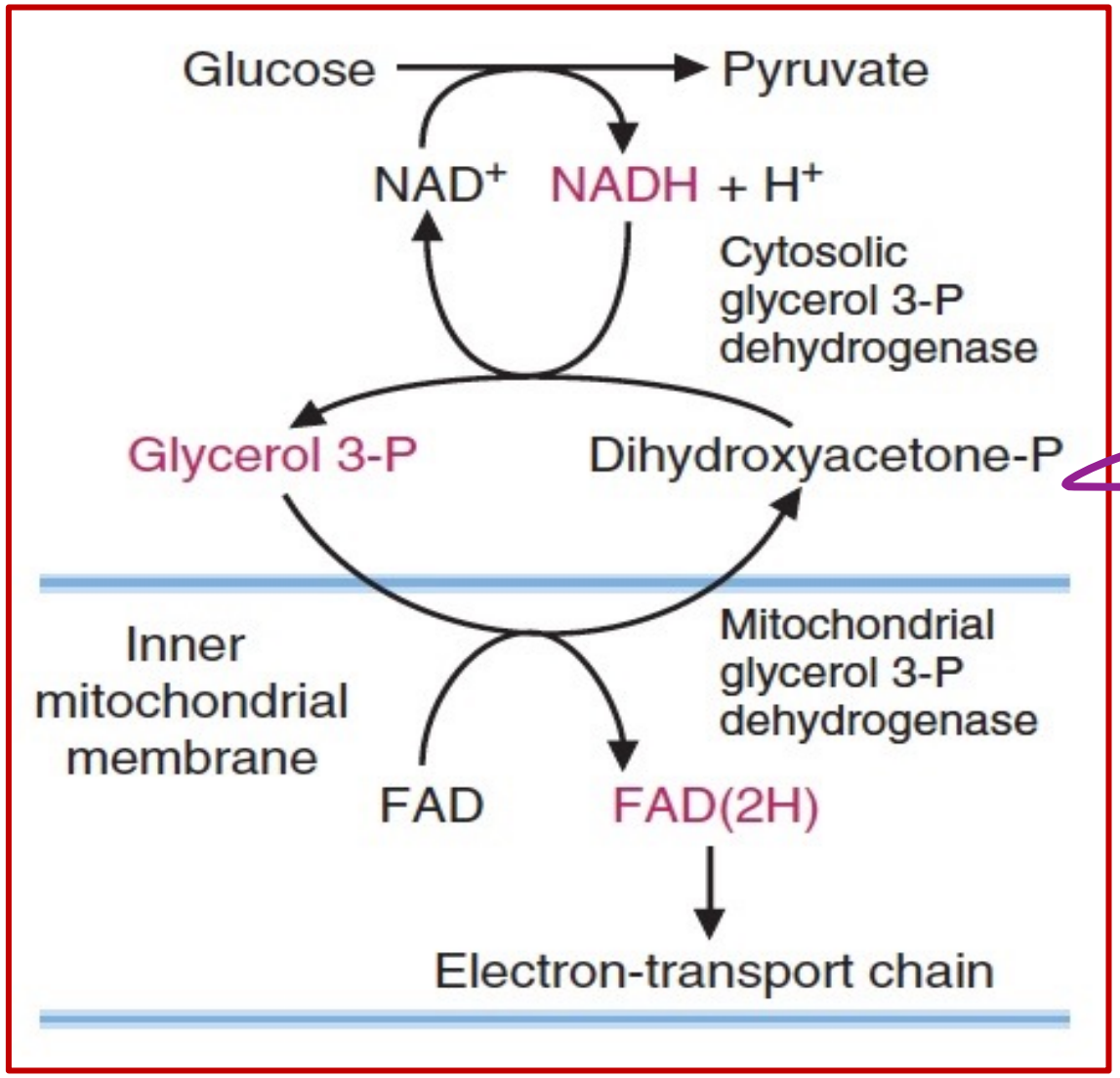
c) Oxaloacetate cannot cross the mitochondrial membrane. In order to return carbon to the cytosol, oxaloacetate is transaminated to aspartate, which can be transported into the cytosol and reconverted to oxaloacetate by another transamination reaction.

d) Note that the transporter for malate requires the opposite movement of α-ketoglutarate; for aspartate the opposite movement of glutamate is required. These are exchange transporters across the inner mitochondrial membrane.

e) In the mitochondrial matrix, each mole of NADH generates approximately 2.5 moles of ATP via oxidative phosphorylation.

f) Because glycolysis produces 2 moles of NADH per mole of glucose, approximately 5 moles of ATP are produced by this shuttle.

NADH Shuttle systems : Glycerol phosphate shuttle



a. Cytosolic DHAP is reduced to glycerol-3-phosphate by NADH.

b. Glycerol-3-phosphate reacts with an FAD-linked dehydrogenase in the inner mitochondrial membrane. DHAP is regenerated and reenters the cytosol.

c. Each mole of $FADH_2$ that is produced generates approximately 1.5 moles of ATP via oxidative phosphorylation.

d. Because glycolysis produces 2 moles of NADH per mole of glucose, approximately 3 moles of ATP are produced by this shuttle.

ENDS..